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Placental Markers of Human Exposure to Polychlorinated Biphenyls and Polychlorinated Dibenzofurans

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Our studies have evaluated biochemical changes in placentae from humans exposed to rice oil contaminated with polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) in Taiwan. Placentae were obtained from nonsmoking women 4 to 5 years after the exposure had occurred. The exposed individuals ingested approximately 1 to 3 g PCBs and 5 mg PCDFs, and many exhibited symptoms characteristic of PCB poisoning. This disease was termed "Yu-Cheng" in Chinese. Based on data from experimental animal models, we examined a number of parameters in placentae from control and exposed women, including arylhydrocarbon hydroxylase (AHH) activity, cytochrome P-450 isozymes, epidermal growth factor (EGF) receptor binding properties and actions, and *Ah* receptor. We also quantified concentrations of various PCB and PCDF congeners known to be present in the contaminated rice oil. Our results revealed a dramatic elevation in placental AHH activity in samples from PCB/PCDF-exposed women. This increase in enzyme activity was associated with a parallel increase in placental microsomal protein immunochemically related to cytochrome P-450 form 6 [derived from 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced rabbit lung]. No other cytochrome P-450 isozyme was detected in placental preparations, and the form 6 homolog was found only in placentae from exposed women. EGF receptor-mediated autophosphorylation capacity was significantly diminished in PCB/PCDF placentae, but this effect was not associated with changes in plasma membrane EGF receptor binding properties (K_d and B_{max}). The EGF receptor autophosphorylation effect correlated well with the decrease in birth weight observed in offspring of exposed women, suggesting that this biochemical event might provide a good marker of effect for the toxic halogenated aromatics. Two PCDF congeners (2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexaCDF) were detected in Yu-Cheng placentae but not controls. Several PCBs were also detected (including the 2,2′,4,4′,5,5′-hexa-CB and 2,3,3′,4,4′,5-hexaCB) in much higher concentrations in Yu-Cheng placentae. Surprisingly, placental concentrations of PCBs correlated better with effects than did the PCDFs. Our findings are discussed in relation to the risk assessment process.

Introduction

During 1979, rice oil contaminated with polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans was accidentally ingested by individuals residing in several counties of Taiwan (*1*). Health officials in Taiwan registered about 1800 individuals with suspected exposure to the contaminated rice oil or symptoms characteristic of PCB poisoning. The rice oil disease was called "Yu-Cheng" in Chinese and was similar to an episode that had occurred in Japan (where it was called "Yusho") in 1968.

Exposed individuals ingested approximately 1 to 3 g PCBs and 5 mg PCDFs during a 3 to 9 month period (*1–3*). Both the PCBs and PCDFs consist of numerous congeners, which vary greatly in their toxicities, as well as in their elimination rates from the body. For example, some congeners exhibit a biological half-life of greater than 50 years, whereas others are in the range of 1 day (*3,4*). Clinical findings for Yu-Cheng and control subjects and their newborns have been abstracted from medical charts and from the local PCB registry records (Table 1). The exposed women in our studies had mild to moderate symptoms typical of clinical effects of exposure to PCBs and PCDFs (*5,6*). These included increased serum activity of aspartate aminotransferase and alanine aminotransferase. Total and direct bilirubin were decreased, and there were large increases in serum triglyceride concentrations. Offspring of women with Yusho or Yu-Cheng tended to be small for gestational age and many had cola-colored skin, swollen eyelids, conjunctival discharge, and pigmented and de-

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Table 1. Clinical findings of Yu-Cheng subjects and their newborns.

Subject ID	Clinical symptom class[a]	Clinical findings		Birth weight, g
		Mother	Newborn	
1	3	Pigmented skin, acne, pimples, abnormal menstrual cycle	Pigmented skin, post-term, otherwise normal	2900
2	1	Pigmented skin	Post-term, otherwise normal	2700
3	3	Pigmented skin, pimples	Post-term, otherwise normal	3000
4	1	Pigmented skin, swelling of eyelids	Normal	2700
5	3	Pigmented skin, acne, otherwise normal	Normal	3000
6	2	Pigmented skin, pimples	Normal	3100
7	1	Pigmented skin, otherwise normal	Normal	2700
8	2	Pigmented skin, pimples	Normal	3100
9	1	Pigmented skin	Normal	2550

[a] Clinical symptom severity progresses from 1 to 4 (*5*). Data taken from (*7,17*).

formed nails (*7*). Some of these effects were transient, whereas others have persisted. The unfortunate poisoning episode in Taiwan provides a unique opportunity to study a human population with high exposure to halogenated aromatics. A better characterization of the halogenated aromatic compounds involved in this episode, as well as some of the biochemical effects resulting from this exposure, would add to the understanding of the potential health hazards arising from exposure to the toxic halogenated aromatics.

The objectives of our studies were to evaluate biochemical changes in placentae from exposed individuals in relation to available information on known effects of the toxic halogenated aromatics in animal models. Parameters measured included placental and blood concentrations of individual PCB and PCDF congeners known to be present in the contaminated rice oil and constituents of the cytochrome P-450-dependent arylhydrocarbon hydroxylase (AHH) activity and concentrations of individual P-450 isozymes. We also evaluated binding capacity and properties of *Ah* receptor and epidermal growth factor (EGF) receptor, including EGF-stimulated autophosphorylation of the EGF receptor. Various biochemical changes were analyzed for positive and negative correlations and data were compared to smoking-related changes in placental biochemistry. We have evaluated utilization of human placenta as a noninvasive method to detect exposure and adverse effects of the toxic halogenated aromatics in hope of enhancing our ability to conduct risk assessments for human exposure to this class of ubiquitous environmental chemicals. Moreover, we also address which congeners are responsible for these effects.

Human Specimens

In collaboration with the Taiwanese government, we obtained placentae from exposed women beginning in February of 1983, or 4 years after the exposure had occurred. Pregnant Yu-Cheng subjects and controls were between 18 and 35 years old and were listed in the PCB registries of either the Taichung or Chang Hua Health Bureaus in Central Taiwan. Clinical symptoms in the exposed women are listed in Table 1. The placental specimens were frozen on dry ice as soon as possible after delivery and transported to the National Institute of Preventive Medicine in Taipei for storage at −70 to −80°C. All specimens were from nonsmoking individuals who were not taking any medications and who had no recognized radiation or chemical exposure. One control subject was selected for each exposed subject; the control subject was the next delivery at the same hospital where the Yu-Cheng subject delivered and was age-matched to within 3 years of the Yu-Cheng subject. For comparison, fresh placentae were obtained from several smoking and nonsmoking volunteers from the obstetrical service at the North Carolina Memorial Hospital.

Placental Metabolism

Halogenated aromatics such as PCBs, PCDFs, and polychlorinated dibenzodioxins (PCDDs) share a number of common physicochemical, biological, and toxic properties. The toxic and biologic potencies of individual PCBs, PCDFs, and PCDDs are structure dependent, with the most active compounds similar in structure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). One of the best studied effects of these compounds in animal models is the induction of some cytochrome P-450-dependent monooxygenases such as AHH (*8–10*).

In our studies, placental homogenates of Yu-Cheng subjects had dramatically elevated levels of AHH activities compared with samples from nonexposed subjects; induction was approximately 100-fold (Fig. 1) (*11*). This finding is similar to the effects of smoking on human placental AHH activity (*12–14*). Since the AHH assay measures chiefly the formation of benzo[a]pyrene (BaP)-phenols, BaP metabolism *in vitro* was studied further by quantifying specific metabolites by high pressure liquid chromatography (HPLC) (*11*). Little or no BaP metabolites were generated in placental homogenates from control subjects. In contrast, placental homogenates from Yu-Cheng subjects generated large amounts of 3-OH-BaP, BaP-quinones, and BaP-7,8-diol. BaP-4,5-diol and BaP-9,10-diol were also produced, but in lesser amounts. These results reveal that effects of these chlorinated chemicals on metabolic processes are highly

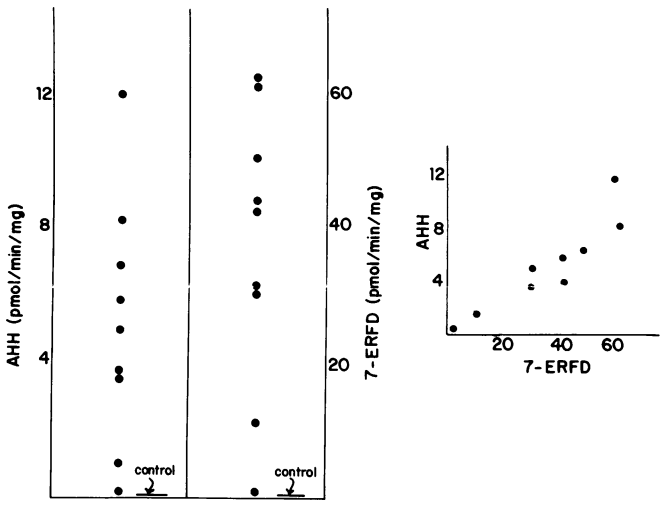

FIGURE 1. Induction of arylhydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin-*O*-deethylase (7-ERFD) in placental microsomes from control and Yu-Cheng individuals. Data summarized from (*17*). Microsomes were prepared and enzyme activities assayed as described in (*17*).

persistent. For comparison, the HPLC profile of BaP metabolites generated by placental homogenates of smokers resembled that of samples from Yu-Cheng subjects (*11*).

Cytochrome P-450 Isozymes

We have used antibodies to rabbit P-450 isozymes to detect homologs of these proteins in human placental microsomes by Western blotting, a technique that couples electrophoretic transfer of protein bands to nitrocellulose paper with immunochemical detection. Treatment of rabbits with 3-methylcholanthrene-type inducers increases the hepatic and extrahepatic concentrations of isozyme 6, a form of cytochrome P-450 that catalyzes the metabolism of BaP and 7-ethoxyresorufin (*15,16*). In this study, we demonstrated the induction of a placental microsomal protein from Yu-Cheng subjects that is immunochemically related to rabbit isozyme 6 (*17*). Concentrations of this isozyme were elevated 150-fold in PCB/PCDF-exposed placentae, and this finding is supported by enzymatic evidence. In contrast, analysis of microsomal samples from control specimens revealed no detectable P-450-related isozyme 6, as well as low AHH and 7-ethoxyresorufin-*O*-deethylase activities (*17*). Among Yu-Cheng subjects, a significant correlation was found between levels of the P-450 isozyme immunologically related to form 6 and AHH ($p < 0.005$) and 7-ethoxyresorufin-*O*-deethylation ($p < 0.01$) activities (*17*). Moreover, there was an excellent correlation between induction of 7-ERFD and AHH activities (Fig. 1) (*17*). The presence of other P-450 isozymes in the crude microsomal preparations from Yu-Cheng and control subjects cannot be ruled out, although no immunoreactive proteins to rabbit isozymes 2 and 5 were detected under our experimental conditions (*17*). Isozyme 6-related protein was also detected in placental microsomes from smokers, but not in samples from nonsmokers, although increases were less than those observed in PCB/PCDF-exposed individuals. Previous studies in the effects of smoking on placental P-450-dependent metabolism using various isozyme antibodies and purified preparations have reported that smoking increases BaP metabolism (*14,18,19*).

Ah Receptor

The initial step in enzyme induction by polycyclic aromatic hydrocarbons appears to involve specific binding of the inducer(s) to a receptor protein. The receptor-ligand complex binds regulatory region(s) of the structural gene for P_1450, leading to the characteristic changes in protein synthesis of P_1450 (*20*). There is a good correlation between induction potencies of individual PCDD, PCDF, and PCB congeners and their receptor binding affinities (*21*). The most active PCB and PCDF compounds are closely related in structure to TCDD. Studies on structure-activity relationship of various classes of halogenated aromatic compounds indicate that the basic requirement for ligand binding to the TCDD or *Ah* receptor is a molecular structure that has a planar rectangle with halogen atoms in the four corners (*9,21*).

For the reason described above, we have analyzed placentae from Yu-Cheng subjects for the presence of *Ah* receptor (*22*). However, the classical *Ah* receptor was not detected in placental cytosolic preparations from either Yu-Cheng or control subjects using ^{3}H-TCDD as the ligand (Table 2). Displaceable TCDD binding was found in placental preparations using the hydroxylapatite binding assay, but this binding was shown by sucrose density gradient analyses to be associated with a binding site(s) distinct from the *Ah* receptor and similar to the 3-methylcholanthrene site observed in rat liver preparations (*23*). This site sediments in the 4*s* region of 5 to 20% sucrose gradients, whereas the classical *Ah* receptor sediments in the 8 to 9*s* region (*10,22*). Moreover, hydroxylapatite adsorption chromatography of radiolabeled placental cytosol revealed that these TCDD binding sites do not correspond to the high-affinity TCDD binding site(s) (*Ah* receptor) found in rat hepatic cytosol (Table 2) (*22*). Studies have suggested that the presence of *Ah* receptor in cytosolic preparations might reflect leakage of nuclear receptor during the preparation of subcellular fractions (*24*), raising the possibility that the *Ah* receptor is located in the nuclear fraction of placental preparations and is not detectable in the cytosol. However, our studies on subcellular localization of receptor show that unoccupied *Ah* receptor is not present in the nuclear fraction from either control or Yu-Cheng subjects (*22*).

The apparent lack of classical *Ah* receptor in placental cytosolic and nuclear extracts of Yu-Cheng and control subjects, coupled with our observation of marked elevation of microsomal AHH activity in placental preparations from only Yu-Cheng subjects, was surprising.

Table 2. *Ah* receptor studies in placentae of Yu-Cheng and control subjects.

Subject	BaP hydroxylase, pmole/mm/mg protein[a]	^{3}H-TCDD binding, fmole/mg protein[b]	Sucrose gradient analysis[c] 4–5*s*	Sucrose gradient analysis[c] 8–9*s*	Analysis of binding on hydroxyapatite columns, potassium phosphate elution[d] 25 mM	110 mM	250 mM	Presence of nuclear binding sites[e]
Control								
1	0.1	1.0	+	–	–	–	+	–
2	0.1	0.7	+	–				–
3	0.1	0.6	+	–				–
4	0.1	0.4	+	–				–
5	0.1	1.0	+	–				–
6	0.1	0.7	+	–				–
7	0.1	0.9	+	–				–
8	0.1	0.7	+	–				–
9	0.1	0.7	+	–				–
Average ± SE	0.1 ± 0	0.8 ± 0.1						
Exposed								
1	3.5	1.2	+	–	–	–	+	–
2	4.5	1.1	+	–				–
3	6.8	0.6	+	–				–
4	4.8	0.4	+	–				–
5	13.6	0.6	+	–				–
6	0.2	1.0	+	–				–
7	3.3	0.6	+	–				–
8	1.0	0.4	+	–				–
9	6.8	0.4	+	–				–
Average ± SE	4.9 ± 1.3	0.7 ± 0.1						
Rat liver[f]	ND	58 ± 6.4	+	+	+	–	–	+

[a] Data summarized from (*22*) was obtained from microsomes prepared from control and exposed placentae.
[b] Data summarized from (*22*) was obtained from cytosolic preparations.
[c] Data summarized from (*22*) and indicate the presence or absence of ^{3}H-TCDD displaceable binding in the 4–5*s* or 8–9*s* regions of 5–20% sucrose gradients.
[d] Data summarized from (*22*) and indicate the concentration of potassium phosphate that elutes displaceable ^{3}H-TCDD displaceable binding from hydroxylapatite columns. Analysis conducted on pooled samples.
[e] Methods for detecting unoccupied *Ah* receptor in nuclear preparations are detailed in (*22*).
[f] Summary data (*22*) on ^{3}H-TCDD binding to rat liver cytosolic preparations.

We offer several possibilities to explain the data (*22*). One is that the placental *Ah* receptor is very labile and is degraded either during sample preparation or during storage of the tissue. A second possibility is that the cytochrome P-450 induction process in human placenta might be associated with a different receptor(s) or a nonreceptor mechanism. Third, it is possible that placental receptor concentrations are below the limits of detection of available methodology. Cytosol prepared from fresh, nonfrozen placentae collected from birth clinics in North Carolina immediately after delivery and assayed for TCDD binding exhibited binding capacities similar to those for samples prepared from these same tissues after storage for several weeks at −80°C. Proteolysis of placental *Ah* receptors during sample preparation is possible, but cytosols prepared either in the presence or in the absence of various protease inhibitors exhibited similar binding characteristics. The mediation of AHH induction by a non-*Ah* receptor mechanism, while possible, is not likely. The overwhelming weight of evidence in the literature indicates that induction of AHH activity in response to TCDD and related compounds is mediated by the *Ah* receptor in various mammalian species. In support of this contention is recent work by Manchester et al. (*25*), which revealed that high concentrations of *Ah* receptor are detected in human placenta if several technical modifications are made in the assay procedure.

EGF Receptor

One of the clinical symptoms observed in Yu-Cheng was that offspring of exposed women were small for gestational age (Table 1) (*7,17*). The mechanism for this effect is unclear. One aspect of our studies has focused on EGF receptor in placentae of individuals exposed to contaminated rice oil and the role that this receptor system might play in the toxic actions of the halogenated aromatics. EGF is a potent mitogen *in vitro*. Interactions of EGF with its membrane-bound receptor in a wide variety of tissues and cells leads to activation of the receptor domain associated with tyrosine kinase leading to autophosphorylation of the EGF receptor (*26*). Other studies have demonstrated that EGF receptor is present in high quantities in human placenta, raising the possibility that changes in EGF receptor properties and function might provide markers for altered fetal development.

In our studies, we found the EGF-stimulated receptor autophosphorylation was decreased by approximately 60% in solubilized placental membranes from PCB/PCDF-exposed individuals (Table 3) (*27*). The phosphorylated receptor is visualized by autoradiography and is detected in the 150 to 170 Kd region of 8% polyacrylamide gels. The amount of ^{32}P on the EGF receptor was quantified by cutting out the 150 to 170 Kd bands and measuring radioactivity by liquid scintillation spectrometry. The average amount of phosphorylation was 1229 ± 365 dpm in the Yu-Cheng placentae compared to 2764 ± 420 dpm for controls. This decrease was strongly correlated with the birth weight reduction seen in offspring of the exposed mothers (*27*).

EGF receptor binding kinetics were also evaluated using the same plasma membranes that were used for the EGF receptor phosphorylation studies (*27*). Scatchard analysis of ^{125}I-EGF binding revealed two binding sites (Fig. 2). For example, placental membranes from control individuals exhibited a high-affinity site (K_d = 0.10 nM, 788 fmole/mg protein) and a lower affinity site (K_d = 17.4 nM, 62 pmole/mg protein). Binding kinetics of membranes from exposed individuals exhibited similar ^{125}I-EGF binding kinetics (Table 3). However, there was a great deal of interindividual variability in the binding properties. The reason for this variation is not clear, but it probably does not reflect differences in concentrations of PCB and PCDF congeners in placentae as discussed in the section of this paper, "Markers of Exposure and Correlations Between Different Parameters." Furthermore, there was no correlation between EGF binding kinetics (K_d and B_{max}) and EGF receptor autophosphorylation among control and/or exposed individuals. This dissociation between EGF binding and receptor autophosphorylation is consistent with a previous study that investigated EGF binding kinetics and EGF-stimulated receptor autophosphorylation capacity in A431 carcinoma cells (*28*).

The mechanism responsible for altered EGF-receptor kinase activity in placental membranes from Yu-Cheng subjects remains unclear. However, it is known that halogenated aromatics modify the EGF receptor.

Table 3. Human placental EGF-stimulated receptor autophosphorylation, EGF-receptor binding kinetics, and birth weight of Yu-Cheng and control subjects.[a]

	^{125}I-EGF receptor binding kinetics[b]		EGF-stimulated phosphorylation,	
Subject	K_d, nM	B_{max}, fmole/mg	dpm per 150–170 kd band	Birth weight, kg
Control (n = 8)	0.10 ± 0.02	788 ± 225	2764 ± 420	3.37 ± 0.13
Yu-Cheng (n = 8)	0.11 ± 0.02	784 ± 305	1229 ± 356[c]	2.86 ± 0.07*

[a] Data summarized from (*27*).

[b] K_d and B_{max} of the high affinity EGF binding site were evaluated by Scatchard analysis using binding data obtained from incubation of ^{125}I-EGF with crude placental plasma membranes (*27*).

* Significantly different from control subjects ($p < 0.05$).

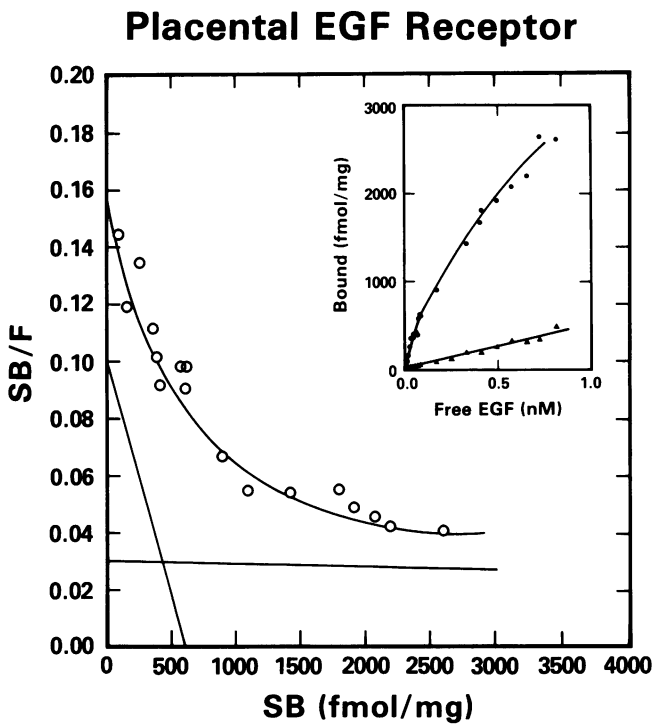

FIGURE 2. Scatchard analysis of [^{125}I]-EGF binding in pooled placental plasma membrane fractions from control individuals. Analysis of binding data using a ligand program (*27*) indicates the presence of two binding sites, one with a K_d of 0.05 nM and the other 6.6 nM.

TCDD decreases EGF binding in rat liver (*29,30*) and in a cultured human keratinocyte cell line (*31*). It has been hypothesized that this effect is mediated through TCDD interactions with the *Ah* receptor (*31*). Another possible mechanism could involve direct interactions of the halogenated aromatics with protein kinase C. The tumor promoter 12-*O*-tetradecanoyl-phorbol-13-acetate decreases EGF binding in Hela cell cultures as a consequence of protein kinase C activation (*32*). In any event, our studies reveal a positive correlation between EGF-stimulated receptor autophosphorylation and decreased birth weight in Yu-Cheng individuals, suggesting that modifications of this pathway might be involved in some of the toxic effects of the contaminated rice oil.

Cigarette smoking also causes disturbances in human placental morphology and metabolic function, as well as decreases in fetal birth weight. We have also investigated the effect of smoking on EGF binding in human placental plasma membrane fractions (Table 4) (*33*). Placentae from smokers had altered high-affinity EGF binding properties with a dissociation constant (K_d = 0.16 ± 0.08 nM; binding capacity, B_{max} = 202 ± 51 fmole/mg), compared to placentae from nonsmokers (K_d = 0.04 ± 0.01 nM; B_{max} = 230 ± 39 fmole/mg). This alteration in K_d was related in a dose-dependent manner to cigarette smoking exposure as measured by maternal

Table 4. Summary of studies on the effects of smoking on human placental EGF receptor binding properties and autophosphorylation capacity.[a]

	Mother's age, years	Gestational age, weeks	Cotinine, ng/mL	Thiocyanate, nmole/mL	^{125}I-EGF binding kinetics: High affinity site K_d, nM	High affinity site B_{max}, fmole/mg	Low affinity site K_d, nM	Low affinity site B_{max}, pmole/mg	EGF-stimulated phosphorylation, dpm per 150–170 kd band
Nonsmokers (n = 11)	30.0 ± 2.2	39.6 ± 0.4	ND	35 ± 4	0.04 ± 0.01	217 ± 41	0.9 ± 0.1	2.6 ± 0.4	1581 ± 464
Smokers (n = 14)	22.2 ± 0.9*	39.1 ± 0.5	110 ± 26*	109 ± 10*	0.16 ± 0.08*	215 ± 62	1.6 ± 0.5	3.6 ± 0.8	1046 ± 228

[a] Values are summarized from (*33*) and indicate mean ± SEM.
* Statistically different from control values ($p < 0.01$).

thiocyanate levels. Smoking did not significantly alter low-affinity EGF receptor binding. Simultaneous analysis of the K_d and B_{max} values for each subject revealed a significant difference between the high-affinity EGF binding kinetics of the nonsmokers compared to the smokers (p = 0.01), while no significant difference was found when comparing the low-affinity EGF binding kinetics between these two sample populations. These findings indicate that exposure of the human placenta to cigarette smoke constituents may result in the alteration of binding properties of the high-affinity EGF receptor, similar to effects reported in animal and *in vitro* models caused by polycyclic aromatic hydrocarbons (*32*). Cigarette smoking also caused a decrease in EGF-stimulated receptor autophosphorylation, but the effect was not as great as that observed in placentae from Yu-Cheng subjects (*33*).

Quantitation of PCDF and PCB Congeners

The analytical procedures used to determine placental concentrations of selected PCB and PCDF congeners were essentially the GC/MS methods of Lundgren et al. (*34*). PCB and PCDF concentrations were quantified by the use of radiolabeled internal standards for estimating recoveries. The PCBs determined were: 2,3,3',4,4'-pentaCB; 2,2',4,4',5'-pentaCB; 2,2',4,4',5,5'-hexaCB; 2,2',3,4,4',5'-hexaCB; 2,3,3',4,4',5-hexaCB; 2,2',3,4,4',5,5'-heptaCB; and 2,2',3,3',4,4',5-heptaCB. The PCDFs determined were: 2,3,4,6,7-pentaCDF; 1,2,4,7,8-pentaCDF; 2,3,4,7,8-pentaCDF, and 1,2,3,4,7,8-hexaCDF. These congeners were selected for analysis because they have been detected in blood and tissues of individuals exposed to PCB-contaminated rice oil (*35*). Two PCDFs were detected in placenta and blood of exposed individuals that were not present in samples from control individuals. These PCDFs were identified as the 2,3,4,7,8-penta and 1,2,3,4,7,8-hexa congeners, and the concentrations in placenta were approximately 0.1 and 0.4 ppb, respectively (*36*). Both congeners are effective binders of the TCDD receptor and their reported EC_{50} values for induction of arylhydrocarbon hydroxylase in rat liver are only slightly less than TCDD (*21*). Our data indicate that the binding affinity of the 2,3,4,7,8-penta congener for the rat hepatic *Ah* receptor was 60% that of TCDD, whereas the corresponding value for the 1,2,3,4,7,8-hexa congener was 9% (*36*). We also quantified the persistent PCBs remaining in the placentas of exposed women and evaluated their binding affinities to the rat liver *Ah* receptor. It should be noted that some of the PCBs present in the contaminated rice oil are not biologically persistent (*3,4*) and would not be present in our placenta and blood samples, as these were obtained 4 to 5 years after the exposure that occurred. A wide variety of PCBs were detected, including 2,2',4,4',5' (1 ppb); 2,3,3',4,4' (1 ppb); 2,2',3',4,4',5' (6 ppb); 2,2',4,4',5,5' (8 ppb); 2,3,3',4,4',5 (2 ppb); 2,2',3,3',4,4',5 (3 ppb); and 2,2',3,4,4',5,5' (2 ppb) (average values in parentheses). None of these compounds are effective binders of the rat liver *Ah* receptor; they are at least three orders of magnitude less efficient than the 2,3,4,7,8-pentaPCDF in displacing TCDD from its receptor (*21,36*).

Analysis of the placental concentrations of PCBs and PCDFs coupled with the *Ah* receptor binding data suggest that the biochemical effects observed in our studies are probably related to the presence of the PCDFs and not the PCBs. It seemed possible to estimate the contribution of each PCDF or PCB congener to the induction of AHH activity in placentae by multiplying the placental concentration of the congener by its relative binding affinity to the *Ah* receptor. Using this approach one can derive an inducing potential for all congeners detected, as well as determine the fraction of the total induction contributed by each congener (Table 5). We estimate that 91% of the induction was caused by the PCDFs and 9% by the PCBs. Furthermore, 60% of the induction appeared to be caused by the 2,3,4,7,8-pentaCDF. Analysis of blood PCDF values from exposed individuals revealed that the 2,3,4,7,8-penta was present at a concentration of approximately 0.015 ppb, compared to 0.05 ppb for the 1,2,3,4,7,8-hexa congener (*34*). There appeared to be a consistent placenta-to-blood ratio of approximately 30 for both congeners in all individuals, suggesting that blood concentrations of PCDFs may be reliable indicators of tissue concentrations in humans.

In addition to the PCBs and PCDFs, placental samples from Yu-Cheng individuals also contained significant concentrations of polychlorinated quaterphenyls

Table 5. Potency of PCDFs and PCBs in placenta of exposed individuals.

		Relative contribution of PCDFs and PCRs in placenta of exposed individuals[a]										Mean ± SD	
		E1		E2		E3		E4		E5			
Compound	EC_{50} equivalence	PI	% Total	PI	% Total	PI	% Total	PI	% Total	PI	% Total	PI	% Total
2,3,7,8-TCDD	100												
2,3,7,8-TCDD	60	6.42		6.24		8.82		6.96		7.02		7.09 ± 1.02	
2,3,4,7,8-PCDF	9	2.81		3.37		3.20		4.01		3.11		3.30 ± 0.45	
Total PCDFs		9.23	92.21	9.61	93.21	12.02	97.00	10.97	87.55	10.13	86.51	10.39 ± 1.12	91.06
2,3,3′,4,4′,5-PCB	0.12	0.13		0.19		0.09		0.45		0.29		0.23 ± 0.14	
2,3,3′,4,4′-PCB	1.10	0.23		0.02		0.02		0.00		0.00		0.05 ± 0.10	
2,2′,4,4′,5,5′-PCB	0.08	0.08		0.13		0.06		0.25		0.19		0.14 ± 0.08	
2,2′,3,3′,4,4′,5-PCB	0	0		0		0		0		0		0	
2,2′,3,4,4′,5-PCB	0	0		0		0		0		0		0	
Total PCBs		0.89	8.79	0.70	6.79	0.37	3.0	1.56	12.45	1.58	13.49	1.02 ± 0.54	8.94
PCDFs + PCBs		10.12		10.31		12.39		12.53		11.71		11.41 ± 1.14	100

[a] Potency index (PI) derived from affinity for rat *Ah* receptor and PCDF and PCB concentrations in placenta of exposed individuals. PI = EC_{50} equivalence (relative binding affinity to *Ah* receptor) × ppb placental PCDF or PCB. E1, E2, E3, E4, E5 each indicate values for a placenta from one exposed individual.

(PCQ) (*36*). GC/MS analysis indicated an average PCQ concentration of approximately 0.5 ppb in exposed individuals, compared to nondetectable concentrations in control samples (limit of detection = 0.05 ppb). Inasmuch as there is virtually no available information on the toxic properties or structure-activity relationships for the PCQs, it is difficult to evaluate their role in the toxic responses to the contaminated rice oil.

Markers of Exposure and Correlations Between Various Parameters

During the course of our studies we have measured numerous parameters in placental samples from a PCB/PCDF-exposed population. It seemed prudent to take advantage of these data by determining possible correlations between different parameters. This approach should provide useful information in our attempts to evaluate the use of animal models in risk assessment and to categorize biochemical events as markers of exposure, markers of effect, or markers of susceptibility. The National Academy of Sciences has developed conceptual definitions for each of these in their recent document, "Biomarkers in Reproductive and Developmental Toxicology" (*37*).

Markers of exposure are biological events that reveal information on external exposure, internal absorbed dose, or dose at the molecular site of toxic action. Within this framework, biologically effective dose can be defined as the amount of chemical (parent compound or active metabolite) at the macromolecular site of action or at a validated surrogate site. In the case of Yu-Cheng individuals, measurement of placental or blood concentrations of PCBs, PCDFs, and PCQs might be considered an internal dose, whereas the amount of chemical bound to the *Ah* receptor would represent the biologically effective dose.

Markers of effect was defined by the National Academy of Sciences as the effects or responses of an organism to a chemical exposure (*37*). Markers of effect should be considered in the context of health impairment or the probability of health impairment. An effect might represent an actual health impairment or disease, an early event in the disease process, or a response peripheral to the disease process but correlated with it and thus predictive of impending health impairment. Effects of PCBs/PCDFs on birth weight and EGF receptor actions could be considered as markers of effect. Within the framework described here it is difficult to categorize some of the responses in Yu-Cheng individuals. For example, aryl hydrocarbon hydroxylase activity is a consequence of the biologically effective dose (*8–10*), but induction of enzyme activity is thought not to be involved in the toxicity of the halogenated aromatics (*38*). Nevertheless it might be considered a good marker of exposure.

Markers of susceptibility are markers which identify individual or population differences related to modifications of toxic responses to environmental chemicals. These markers may reflect intrinsic molecular constituents such as inborn differences in metabolism, immune response, repair capacity, etc. A key issue in understanding or even identifying markers of susceptibility is mechanism of action. Unless we understand how a chemical or class of chemical exerts its toxic actions, it is difficult to effectively use susceptibility markers either in animal models or in human populations.

Positive and negative correlations for the placental markers used in our studies are being published (*27*). The correlations were determined by Pearson Rank Correlation Tables and are expressed as p values. The most significant findings are as follows: AHH activity did not correlate with PCB or PCDF concentrations, but it did correlate with decreased birth weight; and decreased EGF receptor autophosphorylation capacity correlated with decreased birth weight. Decreased

EGF receptor autophosphorylations also correlated with concentrations of total PCBs and the two most predominant PCB congeners in Yu-Cheng placentae (2,2′,4,4′,5,5′- and 2,3′,4,4′,5-hexaCBs). Surprisingly, EGF receptor autophosphorylation did not correlate with placental PCDF concentrations.

These findings suggest that PCB concentrations might be more predictive of toxic effects in human populations than the PCDFs, which, according to experiments in animal models (including *Ah* receptor binding affinities), were the toxic constituents of the contaminated rice oil. We do not have an unequivocal explanation for these findings, but they raise the possibility that non-*Ah* receptor-mediated events may contribute to the toxic effects of the halogenated aromatics in humans. Another possible explanation is that the PCDFs are in fact the prime determinant of the toxic effects but daily variations in tissue-to-blood concentrations of the PCDFs might occur, thereby making it difficult to accurately quantify internal or tissue dose. Dietary or physiological factors that influence release of PCDFs from liver and other tissues may produce transient variations in concentrations of PCDFs in any given tissue.

In parallel experiments, we have also shown that human lymphocytes from Yu-Cheng subjects are more sensitive to the sister-chromatid exchange (SCE)-causing actions of α-naphthoflavone (*in vitro*) than are samples from control subjects (*34*). Similar to our findings in the placental study, the magnitude of the α-naphthoflavone-mediated increase correlates well with serum PCB concentrations but not with PCDF concentrations. In summary, our cumulative studies on biochemical changes in human placentae from a PCB/PCDF exposed population provide an approach to evaluate markers of exposure and markers of effect. These studies indicate that PCB concentrations in tissue and blood may be more reliable predictors of response than are the PCDFs. It seems that human risk assessments for this ubiquitous class of environmental contaminant should attempt to incorporate information presented in this article.

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